

What is claimed is:

1. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

- (1) purification via anion exchange chromatography; and
- (2) purification via cation exchange chromatography following step (1).

2. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

- (1) purification via protein A affinity chromatography;
- (2) purification via anion exchange chromatography following step (1); and
- (3) purification via cation exchange chromatography following step (2).

3. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

- (1) purification via protein A affinity chromatography;
- (2) purification via anion exchange chromatography following step (1);
- (3) purification via cation exchange chromatography following step (2); and
- (4) purification via hydrophobic chromatography following step (3).

4. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to a protein A column equilibrated with a buffer containing sodium phosphate and sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer used for equilibration in step (1) following step (1);

(3) eluting the antibodies with a sodium citrate buffer following step (2);

(4) adjusting the pH of an eluate to 4.0 to 8.5 with a sodium phosphate buffer

following step (3);

(5) adjusting the pH of the eluate of step (4) to 6.0 to 8.5 with a Tris-HCl buffer following step (4);

(6) applying the eluate of step (5) to an anion exchange column equilibrated with a Tris-HCl buffer to bring the eluate into contact with the anion exchange column following step (5);

(7) eluting the antibodies from the anion exchange column with the buffer used for equilibration in step (6) following step (6);

(8) adjusting the pH of the eluate of step (7) to 4.0 to 7.0 with acetic acid or citric acid following step (7);

(9) applying the eluate of step (8) to a cation exchange column equilibrated with a sodium acetate buffer or a sodium phosphate-citrate buffer to bring the eluate into contact with the cation exchange column following step (8);

(10) washing the cation exchange column with the buffer used for equilibration in step (9) following step (9); and

(11) eluting the antibodies from the cation exchange column with a sodium acetate buffer or a sodium phosphate-citrate buffer and a buffer containing sodium chloride following step (10).

5. A method for separating and purifying antibodies from an antibody-containing mixture, which further comprises the steps of:

(12) applying ammonium sulfate to the eluate of step (11) of claim 4 to adjust the pH of the eluate to 6.0 to 8.0 with an aqueous solution of sodium hydroxide following step (11) of claim 4;

(13) applying the eluate of step (12) to a hydrophobic column equilibrated with a buffer containing ammonium sulfate and sodium phosphate to bring the eluate into contact with the hydrophobic column following step (12);

(14) washing the hydrophobic column with the buffer used for equilibration in step (13) following step (13); and

(15) eluting the antibodies from the hydrophobic column by lowering the ammonium sulfate concentration in the buffer of step (13) following step (14).

6. The method according to any one of claims 1 to 5, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

7. The method according to claim 6, wherein the step of virus inactivation is carried out following the step of purification via protein A chromatography.

8. The method according to claim 7, wherein the step of virus inactivation is carried out following step (3) of claim 4 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

9. The method according to any one of claims 6 to 8, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out in the final step of the method for separating and purifying antibodies from an antibody-containing mixture.

10. The method according to any one of claims 4 to 9, wherein a Tris-HCl buffer is used instead of the sodium phosphate buffer of step (4) of claim 4.

11. The method according to any one of claims 4 to 9, wherein the following step is carried out instead of step (4) and step (5) of claim 4:

a step of adjusting the pH of an eluate to 6.0 to 8.5 with a Tris-HCl buffer following step (3).

12. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to a protein A column equilibrated with a buffer containing 10 mM to 100 mM sodium phosphate and 0 M to 4.0 M sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer used for equilibration in step (1) following step (1);

(3) eluting the antibodies with a 10 mM to 100 mM sodium citrate buffer following step (2);

(4) adjusting the pH of an eluate to 4.0 to 8.5 with a 10 mM to 200 mM sodium phosphate buffer following step (3);

(5) adjusting the pH of the eluate of step (4) to 6.0 to 8.5 with a 0.5 M to 1.5 M Tris-HCl buffer following step (4);

(6) applying the eluate of step (5) to an anion exchange column equilibrated with a 10 mM to 20 mM Tris-HCl buffer to bring the eluate into contact with the anion exchange column following step (5);

(7) eluting the antibodies from the anion exchange column with the buffer used for equilibration in step (6) following step (6);

(8) adjusting the pH of the eluate of step (7) to 4.0 to 7.0 with 0.5 M to 1.5 M acetic acid or citric acid following step (7);

(9) applying the eluate of step (8) to a cation exchange column equilibrated with a 10 mM to 50 mM sodium acetate buffer or a 10 mM to 50 mM sodium phosphate-10 mM to 50 mM citrate buffer to bring the eluate into contact with the cation exchange column following step (8);

(10) washing the cation exchange column with the buffer used for equilibration in step (9) following step (9); and

(11) eluting the antibodies from the cation exchange column with a 10 mM to 50 mM sodium acetate or 10 mM to 50 mM sodium phosphate-10 mM to 50 mM citrate buffer and a buffer containing 0.1 M to 1.0 M sodium chloride following step (10).

13. A method for separating and purifying antibodies from an antibody-containing mixture, which further comprises the steps of:

(12) applying ammonium sulfate to the eluate of step (11) of claim 12 to bring the ammonium sulfate concentration thereof to 0.8 M to 1.2 M and adjusting the pH thereof to 6.0 to 8.0 with an aqueous solution of 1.0 M to 10.0 M sodium hydroxide following step (11) of claim 12;

(13) applying the eluate of step (12) to a hydrophobic column equilibrated with a buffer containing 0.8 M to 1.2 M ammonium sulfate and 10 mM to 100 mM sodium phosphate to bring the eluate into contact with the hydrophobic column following step (12);

(14) washing the hydrophobic column with the buffer used for equilibration in step (13) following step (13); and

(15) eluting the antibodies from the hydrophobic column by lowering the ammonium sulfate concentration in the buffer of step (13) following step (14).

14. The method according to claim 12 or 13, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

15. The method according to claim 14, wherein the step of virus inactivation is carried out following step (3) of claim 12 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

16. The method according to claim 14 or 15, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (11) of claim 12.

17. The method according to claim 14 or 15, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (15) of claim 13.

18. The method according to any one of claims 12 to 17, wherein a 0.5 M to 1.5 M Tris-HCl buffer is used instead of the sodium phosphate buffer of step (4) of claim 12.

19. The method according to any one of claims 12 to 17, wherein the following step is carried out instead of step (4) and step (5) of claim 12:

a step of adjusting the pH of an eluate to 6.0 to 8.5 with a 0.5 M to 1.5 M Tris-HCl buffer following step (3).

20. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to a protein A column equilibrated with a buffer containing 10 mM to 20 mM sodium phosphate and 0.15 M sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer used for equilibration in step (1) following step (1);

(3) eluting the antibodies with a 10 mM to 20 mM sodium citrate buffer following step (2);

(4) adjusting the pH of an eluate to 4.0 to 8.5 with a 10 mM to 100 mM sodium phosphate buffer following step (3);

(5) adjusting the pH of the eluate of step (4) to 6.0 to 8.5 with a 1.0 M to 1.5 M Tris-HCl buffer following step (4);

(6) applying the eluate of step (5) to an anion exchange column equilibrated with a 10 mM Tris-HCl buffer to bring the eluate into contact with the anion exchange column following step (5);

(7) eluting the antibodies from the anion exchange column with the buffer used for equilibration in step (6) following step (6);

(8) adjusting the pH of the eluate of step (7) to 4.0 to 7.0 with 1 M acetic acid or citric acid following step (7);

(9) applying the eluate of step (8) to a cation exchange column equilibrated with a 20 mM sodium acetate buffer or a 20 mM sodium phosphate-10 mM citrate buffer to bring the eluate into contact with the cation exchange column following step (8);

(10) washing the cation exchange column with the buffer used for equilibration in step (9) following step (9); and

(11) eluting the antibodies from the cation exchange column with a 20 mM sodium acetate or 20 mM sodium phosphate-10 mM citrate buffer and a buffer containing 0.3 M sodium chloride following step (10).

21. A method for separating and purifying antibodies from an antibody-containing mixture, which further comprises the steps of:

(12) applying ammonium sulfate to the eluate of step (11) of claim 20 to bring the ammonium sulfate concentration thereof to 1.0 M and adjusting the pH thereof to 6.0 to 8.0 with an aqueous solution of 10.0 M sodium hydroxide following step (11) of claim 20;

(13) applying the eluate of step (12) to a hydrophobic column equilibrated with a buffer containing 1.0 M ammonium sulfate and 10 mM sodium phosphate to bring the eluate into contact with the hydrophobic column following step (12);

(14) washing the hydrophobic column with the buffer used for equilibration in step (13) following step (13); and

(15) eluting the antibodies from the hydrophobic column by lowering the ammonium sulfate concentration in the buffer of step (13) following step (14).

22. The method according to claim 20 or 21, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to

stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

23. The method according to claim 22, wherein the step of virus inactivation is carried out following step (3) of claim 20 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

24. The method according to claim 22 or 23, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (11) of claim 20.

25. The method according to claim 22 or 23, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (15) of claim 21.

26. The method according to any one of claims 20 to 25, wherein a 1.0 M Tris-HCl buffer is used instead of the sodium phosphate buffer of step (4) of claim 20.

27. The method according to any one of claims 20 to 25, wherein the following step is carried out instead of step (4) and step (5) of claim 20:

a step of adjusting the pH of an eluate to 6.0 to 8.5 with a 1.0 M Tris-HCl buffer following step (3).

28. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to a protein A column equilibrated with a buffer containing 10 mM to 20 mM sodium phosphate and 0.15 M sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer used for equilibration in step (1) following step (1);

(3) eluting the antibodies with a 10 mM to 20 mM sodium citrate buffer following step (2);

(4) adjusting the pH of an eluate to 7.0 with a 10 mM to 100 mM sodium phosphate buffer following step (3);

(5) adjusting the pH of the eluate of step (4) to 8.0 with a 1.0 M to 1.5 M Tris-HCl buffer following step (4);

(6) applying the eluate of step (5) to an anion exchange column equilibrated with a 10 mM Tris-HCl buffer to bring the eluate into contact with the anion exchange column following step (5);

(7) eluting the antibodies from the anion exchange column with the buffer used for equilibration in step (6) following step (6);

(8) adjusting the pH of the eluate of step (7) to 5.0 with 1.0 M acetic acid or citric acid following step (7);

(9) applying the eluate of step (8) to a cation exchange column equilibrated with a 20 mM sodium acetate buffer or a 20 mM sodium phosphate-10 mM citrate buffer to bring the eluate into contact with the cation exchange column following step (8);

(10) washing the cation exchange column with the buffer used for equilibration in step (9) following step (9); and

(11) eluting the antibodies from the cation exchange column with a 20 mM sodium acetate or 20 mM sodium phosphate-10 mM citrate buffer and a buffer containing 0.3 M sodium chloride following step (10).

29. A method for separating and purifying antibodies from an antibody-containing mixture, which further comprises the steps of:

(12) adding ammonium sulfate to the eluate of step (11) of claim 28 to bring the ammonium sulfate concentration thereof to 1.0 M and adjusting the pH thereof to 7.0 with an aqueous solution of 10.0 M sodium hydroxide following step (11) of claim 28;

(13) applying the eluate of step (12) to a hydrophobic column equilibrated with a buffer containing 1.0 M ammonium sulfate and 10 mM sodium phosphate to bring the eluate into contact with the hydrophobic column following step (12);

(14) washing the hydrophobic column with the buffer used for equilibration in step (13) following step (13); and

(15) eluting the antibodies from the hydrophobic column by lowering the ammonium sulfate concentration in the buffer of step (13) following step (14).

30. The method according to claim 28 or 29, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

31. The method according to claim 30, wherein the step of virus inactivation is carried out following step (3) of claim 28 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

32. The method according to claim 30 or 31, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (11) of claim 28.

33. The method according to claim 30 or 31, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (15) of claim 29.

34. The method according to any one of claims 28 to 33, wherein a 1.0 M Tris-HCl buffer is used instead of the sodium phosphate buffer of step (4) of claim 28.

35. The method according to any one of claims 28 to 33, wherein the following step is carried out instead of step (4) and step (5) of claim 28:

a step of adjusting the pH of an eluate to 8.0 with a 1.0 M Tris-HCl buffer following step (3).

36. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to a protein A column equilibrated with a buffer of pH 6.0 to 8.5 to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer of pH 6.0 to 8.5 following step (1);

(3) eluting the antibodies with a buffer of pH 2.7 to 4.0 following step (2);

(4) adjusting the pH of an eluate to 4.0 to 8.5 following step (3);

(5) adjusting the pH of the eluate of step (4) to 6.0 to 8.5 following step (4);

(6) applying the eluate of step (5) to an anion exchange column equilibrated with a buffer of pH 6.0 to 8.5 to bring the eluate into contact with the anion exchange column following step (5);

(7) eluting the antibodies from the anion exchange column with the buffer of pH 6.0 to 8.5 following step (6);

(8) adjusting the pH of the eluate of step (7) to 4.0 to 7.0 following step (7);

(9) applying the eluate of step (8) to a cation exchange column equilibrated with a buffer of pH 4.0 to 7.0 to bring the eluate into contact with the cation exchange column following step (8);

(10) washing the cation exchange column with a buffer of pH 4.0 to 7.0 following step (9); and

(11) eluting the antibodies from the cation exchange column with a buffer of pH 4.0 to 7.0 with a salt concentration of 0.1 M to 1.0 M following step (10).

37. A method for separating and purifying antibodies from an antibody-containing mixture, which further comprises the steps of:

(12) applying salt to the eluate of step (11) of claim 36 to bring the salt concentration thereof to 0.8 M to 1.2 M and adjusting the pH thereof to 6.0 to 8.0;

(13) applying the eluate of step (12) to a hydrophobic column equilibrated with a buffer of pH 6.0 to 8.0 containing the salt of step (12) at 0.8 M to 1.2 M to bring the eluate into contact with the hydrophobic column;

(14) washing the hydrophobic column with the buffer of pH 6.0 to 8.0 containing the salt of step (12) at 0.8 M to 1.2 M; and

(15) eluting the antibodies from the hydrophobic column by lowering the salt concentration in the buffer of step (13).

38. The method according to claim 36 or 37, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

39. The method according to claim 38, wherein the step of virus inactivation is carried out following step (3) of claim 36 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

40. The method according to claim 38 or 39, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (11) of claim 36.

41. The method according to claim 38 or 39, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (15) of claim 37.

42. The method according to claim 36, wherein the buffer of pH 6.0 to 8.5 used in step (2) is identical to the buffer used in step (1), the buffer of pH 6.0 to 8.5 used in step (7) is identical to the buffer used in step (6), and the buffer of pH 4.0 to 7.0 used in step (10) is identical to the buffer used in step (9).

43. The method according to any one of claims 37 to 42, wherein the buffer of pH 6.0 to 8.0 containing the salt of step (12) used in step (14) of claim 37 at 0.8 M to 1.2 M is identical to the buffer used in step (13).

44. The method according to any one of claims 36 to 43, wherein the salt used in step (11) of claim 36 is sodium chloride.

45. The method according to any one of claims 36 to 44, wherein the salt used in step (12) of claim 37 is ammonium sulfate.

46. The method according to any one of claims 36 to 45, wherein the salt concentration of the buffer used in step (1) of claim 36 is 4.0 M or lower.

47. The method according to claim 46, wherein the salt is sodium chloride.

48. A method for separating and purifying antibodies from an antibody-containing mixture comprising at least the following steps of:

(1) applying an antibody-containing mixture to an anion exchange column equilibrated with a buffer of pH 6.0 to 8.5 to bring the mixture into contact with the anion exchange column;

(2) eluting the antibodies from the anion exchange column with a buffer of pH 6.0 to 8.5 following step (1);

(3) adjusting the pH of the eluate of step (2) to 4.0 to 7.0 following step (2);

(4) applying the eluate of step (3) to a cation exchange column equilibrated with a buffer of pH 4.0 to 7.0 to bring the eluate into contact with the cation exchange column following step (3);

(5) washing the cation exchange column with a buffer of pH 4.0 to 7.0 following step (4); and

(6) eluting the antibodies from the cation exchange column with a buffer of pH 4.0 to 7.0 with a salt concentration of 0.1 M to 1.0 M following step (5).

49. The method according to claim 48, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

50. The method according to claim 49, wherein the step of virus inactivation is carried out before step (1) of claim 48 by adjusting the pH of the antibody-containing solution to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

51. The method according to claim 49 or 50, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out following step (6) of claim 48.

52. The method according to any one of claims 48 to 51, wherein the buffer of pH 6.0 to 8.5 used in step (2) of claim 48 is identical to the buffer used in step (1) and the buffer of pH 4.0 to 7.0 used in step (5) of claim 48 is identical to the buffer used in step (4).

53. The method according to any one of claims 48 to 52, wherein the salt used in step (6) of claim 48 is sodium chloride.

54. The method according to any one of claims 1 to 53, wherein the antibodies are human antibodies.

55. The method according to any one of claims 1 to 54, wherein the antibodies are monoclonal antibodies.

56. The method according to any one of claims 1 to 55, wherein the antibodies are IgG antibodies.

57. The method according to claim 56, wherein the antibodies are IgG1, IgG2, or IgG4 antibodies.

58. The method according to any one of claims 54 to 57, wherein the antibodies have an amino acid sequence derived from the amino acid sequence of the heavy chain constant region by deletion, substitution, or addition of at least one amino acid residue in the naturally occurring heavy chain constant region.

59. The method according to any one of claims 54 to 58, wherein the antibodies are covalently or coordinately bound to other compounds.

60. A method for separating human antibodies from ungulate antibodies via protein A affinity chromatography from a mixture containing human antibodies and ungulate antibodies.

61. The method according to claim 60, wherein the ungulate is selected from the group consisting of a bovine, a goat, and a sheep.

62. The method according to claim 60 or 61, wherein separation is carried out via pH gradient elution.

63. The method according to claim 60 or 61, wherein separation is carried out via pH stepwise elution.

64. A method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies comprising at least the following steps of:

(1) applying a mixture containing human antibodies and ungulate antibodies to a protein A column equilibrated with a buffer containing disodium phosphate, sodium acetate, glycine, and sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer of step (1) following step (1);
and

(3) eluting the human antibodies from the protein A column by lowering the pH of the buffer of step (1) following step (2).

65. The method according to claim 64, wherein the ungulate is selected from the group consisting of a bovine, a goat, and a sheep.

66. The method according to claim 64 or 65, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

67. The method according to claim 66, wherein the step of virus inactivation is carried out following step (3) of claim 64 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

68. The method according to claim 66 or 67, wherein the step of virus removal

via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out so as to comprise the final step of the method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies.

69. The method according to any one of claims 64 to 68, wherein the pH level is lowered in a gradient manner.

70. The method according to any one of claims 64 to 68, wherein the pH level is lowered stepwise.

71. A method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies comprising at least the following steps of:

(1) applying a mixture containing human antibodies and ungulate antibodies to a protein A column equilibrated with a buffer containing 0.05 M to 0.15 M disodium phosphate, 0.05 M to 0.15 M sodium acetate, 0.05 M to 0.15 M glycine, and 0.05 M to 0.20 M sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer of step (1) following step (1);
and

(3) eluting the human antibodies from the protein A column by lowering the pH of the buffer of step (1) following step (2).

72. The method according to claim 71, wherein the ungulate is selected from the group consisting of a bovine, a goat, and a sheep.

73. The method according to claim 71 or 72, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

74. The method according to claim 73, wherein the step of virus inactivation is carried out following step (3) of claim 71 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

75. The method according to claim 73 or 74, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out so as to comprise the final step of the method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies.

76. The method according to any one of claims 71 to 75, wherein the pH level is lowered in a gradient manner.

77. The method according to any one of claims 71 to 75, wherein the pH level is lowered stepwise.

78. A method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies comprising at least the following steps of:

(1) applying a mixture containing human antibodies and ungulate antibodies to a protein A column equilibrated with a buffer containing 0.10 M disodium phosphate, 0.10 sodium acetate, 0.10 M glycine, and 0.15 M sodium chloride to bring the mixture into contact with the protein A column;

(2) washing the protein A column with the buffer of step (1) following step (1);
and

(3) eluting the human antibodies from the protein A column by lowering the pH of the buffer of step (1) following step (2).

79. The method according to claim 78, wherein the ungulate is selected from the group consisting of a bovine, a goat, and a sheep.

80. The method according to claim 78 or 79, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

81. The method according to claim 80, wherein the step of virus inactivation is carried out following step (3) of claim 78 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

82. The method according to claim 80 or 81, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out so as to comprise the final step of the method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies.

83. The method according to any one of claims 78 to 82, wherein the pH level is lowered in a gradient manner.

84. The method according to any one of claims 78 to 82, wherein the pH level is lowered stepwise.

85. A method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies comprising at least the following steps of:

(1) applying a mixture containing human antibodies and ungulate antibodies to a protein A column equilibrated with a buffer of pH 7.5 to 8.5 to bring the mixture into contact with the protein A column;

(2) washing the protein A column with a buffer of pH 7.5 to 8.5; and

(3) eluting the human antibodies from the protein A column by lowering the pH.

86. The method according to claim 85, wherein the ungulate is selected from the group consisting of a bovine, a goat, and a sheep.

87. The method according to claim 85 or 86, which further comprises one or more of the steps of: virus inactivation by allowing the antibody-containing solution to stand under acidic conditions; virus removal from the antibody-containing solution with the use of a virus removal filter; and aseptic filtration of the antibody-containing solution.

88. The method according to claim 87, wherein the step of virus inactivation is carried out following step (3) of claim 85 by adjusting the pH of the eluate obtained in step (3) to 4 or lower and allowing the eluate to stand for 30 minutes or longer.

89. The method according to claim 87 or 88, wherein the step of virus removal via filtration with the use of a virus removal filter and/or the step of aseptic filtration are carried out so as to comprise the final step of the method for isolating human antibodies from a mixture containing human antibodies and ungulate antibodies.

90. The method according to any one of claims 85 to 89, wherein the pH level is lowered in a gradient manner.

91. The method according to any one of claims 85 to 89, wherein the pH level is lowered stepwise.

92. The method according to any one of claims 85 to 91, wherein the buffer of pH 7.5 to 8.5 used in step (2) of claim 85 is identical to the buffer used in step (1).

93. The method according to any one of claims 85 to 92, wherein a salt

concentration of the buffer of pH 7.5 to 8.5 used in step (1) of claim 85 is 0.05 M to 0.20 M.

94. The method according to claim 93, wherein the salt is sodium chloride.

95. The method according to any one of claims 60 to 94, wherein the human antibodies are human polyclonal antibodies.

96. The method according to any one of claims 60 to 95, wherein the ungulate antibodies are selected from the group consisting of bovine polyclonal antibodies, goat polyclonal antibodies, and sheep polyclonal antibodies.

97. The method according to any one of claims 60 to 96, wherein the human antibodies are IgG antibodies.

98. The method according to claim 97, wherein the human antibodies are IgG1 antibodies, IgG2 antibodies, or IgG4 antibodies.